

PTEN (138G6) Rabbit mAb (Alexa Fluor® 647 Conjugate)

500 µl
 (50 tests)

Orders ■ 877-616-CELL (2355)
 orders@cellsignal.com
Support ■ 877-678-TECH (8324)
 info@cellsignal.com
Web ■ www.cellsignal.com

rev. 06/09/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Entrez-Gene ID #5728
Swiss-Prot Acc. #P60484

Applications	Species Cross-Reactivity	Source	Isotype
F Endogenous	H, M, R, Mk	Rabbit	IgG

Description: Cell Signaling Technology antibody is conjugated to Alexa Fluor® 647 fluorescent dye and tested in-house for direct flow cytometric analysis of human cells.

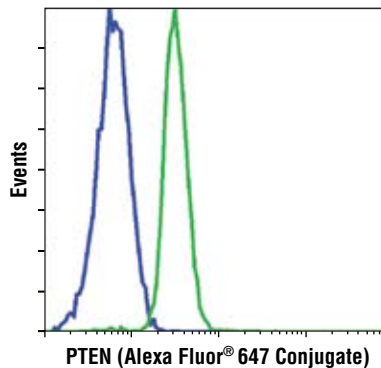
*The unconjugated antibody #9559 reacts with human, monkey, mouse and rat PTEN protein. CST expects that PTEN (138G6) Rabbit mAb (Alexa Fluor® 647 Conjugate) will also recognize PTEN in these species.

Background: PTEN (phosphatase and tensin homologue deleted on chromosome ten), also referred to as MMAC (mutated in multiple advanced cancers) phosphatase, is a tumor suppressor implicated in a wide variety of human cancers (1). PTEN encodes the 403 amino acid polypeptide originally described as a dual-specificity protein phosphatase (2). The main substrates of PTEN are inositol phospholipids generated by the activation of the phosphoinositide 3-kinase (PI3K) (3). PTEN is a major negative regulator of the PI3K/Akt signaling pathway (1,4-5). PTEN possesses a carboxy-terminal noncatalytic regulatory domain containing three phosphorylation sites (Ser380, Thr382 and Thr383), which regulates its stability and may play an important role in control of its biological activity (6,7). PTEN also regulates p53 protein levels and activity (8) and is involved in G protein coupled signaling during chemotaxis (9,10).

Specificity/Sensitivity: PTEN (138G6) Rabbit mAb (Alexa Fluor® 647 Conjugate) detects endogenous levels of total PTEN protein.

Source/Purification: Monoclonal antibody is produced by immunizing rabbits with a synthetic peptide (KLH-coupled) derived from the carboxy-terminal sequence of human PTEN. The antibody was conjugated to Alexa Fluor® 647 under optimal conditions with an F/P ratio of 2-5. The Alexa Fluor® 647 dye is maximally excited by red light (e.g. 633 nm He-Ne laser). Antibody conjugates of the Alexa Fluor® 647 dye produce bright far-red-fluorescence emission, with a peak at 665 nm.

Directions for Use: Add 10 µl of the conjugated antibody to 500,000 cells in 90 µl PBS/0.5% BSA. See protocol for more details.



Flow cytometric analysis of PTEN-negative Jurkat cells (blue) versus PTEN-positive SEM cells (green), using PTEN (138G6) Rabbit mAb (Alexa Fluor® 647 Conjugate).

Background References:

- (1) Cantley, L.C. and Neel, B.G. (1999) *Proc. Natl. Acad. Sci. USA* 96, 4240–4245.
- (2) Myers, M.P. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 9052–9057.
- (3) Myers, M.P. et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 13513–13518.
- (4) Wan, X. and Helman, L.J. (2003) *Oncogene* 22, 8205–8211.
- (5) Wu, X. et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 15587–15591.
- (6) Vazquez, F. et al. (2000) *Mol. Cell. Biol.* 20, 5010–5018.
- (7) Torres, J. and Pulido, R. (2001) *J. Biol. Chem.* 276, 993–998.
- (8) Freeman, D.J. et al. (2003) *Cancer Cell* 3, 117–130.
- (9) Funamoto, S. et al. (2002) *Cell* 109, 611–623.
- (10) Iijima, M. and Devreotes, P. (2002) *Cell* 109, 599–610.

Storage: Supplied in PBS (pH 7.2), less than 0.1% Sodium azide, 2 mg/ml BSA. Store at 4°C. Protect from light. Do not freeze.

Recommended Antibody Dilutions:
 Flow Cytometry 1:10

Companion Products:

PTEN Blocking Peptide #1250
 Phospho-Akt (Ser473) (193H12) Rabbit mAb (Alexa Fluor® 488 Conjugate) #2336

The Alexa Fluor® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc., for research use only, except for use in combination with DNA microarrays. The Alexa Fluor® dyes (except for Alexa Fluor® 430 dye) are covered by pending and issued patents.
 Alexa Fluor® is a registered trademark of Molecular Probes, Inc.

Flow Cytometry Protocol for Intracellular Staining Using Conjugated Primary Antibodies

A Solutions and Reagents

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 ml distilled water (dH_2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100ml 1X PBS. Store at 4°C

B Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
3. Fix for 10 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

C Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at -20°C in 90% methanol.

D Staining Using Conjugated Primary Antibodies

NOTE: Allow for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemacytometer or alternative method.

1. Aliquot 5×10^5 cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation.
3. Resuspend cells in 90 μl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add 10 μl of conjugated antibody to the assay tubes.
6. Incubate for 30-60 minutes, in the dark at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.